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EFFECT OF ANILIN IN RABBITS *

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The effect of anilin on human beings has been noted in a limited number of cases of industrial anilin poisoning. Malden,[1] in 1907, made a study of the blood of 13 men poisoned by anilin, working in the dye industry; Krause[2] described 2 cases; Trespe,[3] 2 cases; and more recently Luce and Hamilton[4] have reported cases in the dye and rubber industry, and Hudson[5] in the manufacture of explosives. Price, Jones, and Boycott[6] studied the blood and marrow of rabbits, in experimental anilin poisoning. It was thought interesting, during the course of an experiment on the effect of anilin on the production of antibodies in rabbits, to follow from day to day the changes in the blood count, to study the blood picture in stained smears, and also the sections of different tissues after death.

The anilin used was as pure as it was possible to make it, and was injected subcutaneously without being dissolved or diluted in anything. The leucocyte count was unaffected by the injection of anilin. In 1 set of 7 rabbits, injected with 0.2 c.c. on 4 successive days, whose leukocytes were counted every other day, the count never varied more than 3000 cells from what it was before the injections were begun. Another set of older rabbits which received larger doses, 0.8 c.c. on 3 successive days, gave the same results, although the red count was much reduced—in 1 case to 550,000. These results confirm the findings of Hudson but not those of Malden, who found a leukocytosis in 8 of 13 of his human cases.

Red counts were made every other day in the set of rabbits injected with 0.2 c.c. on 4 successive days. There was a rapid diminution in the red cells: 0.2 c.c. produced no appreciable reduction, 0.4 c.c., a reduction of about 1 million per c.c., and 0.8 c.c., a reduction of 2 or

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[1] Jour. Hyg., 1907, 7, p. 672.

[2] Med. Klin., 1908, 55, p. 10.

[3] München. med. Wchnschr., 1911, 58, p. 1720.

[4] Jour. Am. Med. Assn., 1916, 66, p. 1441.

[5] Med. Rec., 1917, 91, p. 89.

[6] Guy's Hosp. Rep., 1909, 63, p. 309.

3 million. In the animals that survived for any time after the injections were stopped, there was a gradual return toward normal. In one case, on the 8th day after the last injection, the count had risen from 1,970,000 to 2,860,000; in another from 1,310,000 to 2,670,000. None of the animals survived long enough to show how much time a complete recovery would take.

The differential counts failed to vary strikingly from normal. There was a slight increase in the polymorphonuclear leukocytes during the course of the injections in every case, but in none was it more than a 7% increase above the normal taken from the counts before the injections began. The eosinophils and basophils showed no appreciable variation. These results are somewhat surprising in view of Malden's findings in human blood, where in 5 of 9 cases there was a reduction in the polymorphonuclear cells and an increase in the mononuclears.

The morphologic changes in the red cells were much more striking. In every case there was marked metachromasia. It began to appear after the injection of 0.4 c.c., and increased till the full dose of 1 c.c. had been given. It gradually disappeared after the injections were stopped, and was hardly noticeable on the 10th day after the last injection. Great variation in size followed closely the course of the metachromasia, the cells ranging from 3-10 microns, the larger ones being the more numerous. Most of the cells averaged a little over 7 microns, as compared with 6 microns in the normal. Nucleated reds were occasionally found, 1 or 2 in the course of a differential count. Every animal showed stippling of the red cells. In some cases there were 3 or 4 stippled cells in a field and in others on the same day, after the same dose, a careful search of many fields had to be made. It was never found after 0.2 c.c. had been given, and in only 1 of 5 cases after 0.4 c.c. It appeared in all after 0.8 c.c. and 1.0 c.c. Five days after the last injection it was found in only 1 of 7 cases.

The changes in the tissues were studied after small doses of 0.2 c.c. for each of 2 days, medium doses, that is 0.2 c.c., for 4 or 5 successive days, and large doses, 0.8 c.c., every other day till the animals died. The only macroscopic changes in any of these rabbits were in the liver and spleen. In some cases after small and medium doses, and in every case after large doses, the liver was deeply bile-stained and spotted with multiple gray areas the size of an ordinary pin head. The spleen was generally a deeper red than normal, sometimes enlarged, and in

1 animal, which had received 2.4 c.c. of anilin in all, the spleen was 3 or 4 times the normal size.

Sections were made of kidney, liver, spleen, bone marrow of the femur, in a few instances of the marrow of all the long bones, and of the brain and spinal cord at different levels. The kidney, brain, and cord showed no changes. The liver, in over 50% of the animals, showed microscopically small areas of focal necrosis with some round cell infiltration, an occasional giant cell, some karyorrhexis in the center of the area, and the whole area walled off by a new growth of connective tissue. This necrosis was seen after small, medium, and large doses, as early as the 3rd day after the injection, and as late as the 21st day. In a few cases, the liver cords were shrunken, and in about one third, there were degeneration and edema of the liver cells around the central vein, and slight fatty changes. In the spleen, in all cases, there was an increase in the number of pigment cells. In some cases, the spleen was engorged with blood. That the spleen may play an important part in the toxic effect of anilin is suggested by the resistance shown by an old splenectomized rabbit, which stood 6.4 c.c. of anilin given in doses of 0.8 c.c. in the course of 12 days, nearly 3 times as much as any other animal stood.

The bone marrow of the femur was studied carefully in sections and in smears. The smears were treated with alphanaphthol and pyronin, according to the method of Graham,[7] to discover whether the myelocytes of the marrow, the red cells, or the nongranular cells were more affected. The changes in the marrow were very marked. Injections of as small amounts as 0.2 c.c. were followed in as short a time as 2 days by a marked hyperplasia, which replaced nearly all the fat. Larger doses produce solidly cellular marrows, which continued fairly cellular until 9 or 10 days after the injections were stopped. The fat did not return to any extent when the hyperplasia passed away, and its place was taken by a homogeneous substance. From a study of the sections and smears, this hyperplasia seems due to an increase in the normoblasts and premyelocytes, or nongranular cells. The giant cells were not markedly increased and showed no particular phagocytosis of leukocytes, as do those in the marrow of toluene rabbits.[8] The kinetic figures were not noticeably increased.

As normal rabbits at necropsy may show lesions of the liver simulating those found after anilin injections, guinea-pigs which less commonly show such lesions, were injected subcutaneously with 0.1 c.c. of anilin for 4 days, and the red and white cells counted. As the red count was not affected by this dose, and the only reaction seemed to be a local induration of the skin and subsequent ulceration, the dose was increased to 0.2 c.c. for 2 days, then 0.4 c.c. for 2 days, and finally 0.6 c.c. for 2 days, when the animals died. In all, each of these 3 pigs received 2.8 c.c. of anilin. The ulcers at the site of inoculation became worse, but the red count was not affected, and the stippling, anisocytosis, and metachromasia were slight. At necropsy, one animal showed focal necrosis of the liver in every way like that found in half the rabbits. The other 2 showed no changes in the liver. The spleen in all 3 was filled with pigment cells.

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[7] Jour. Med. Research, 1916, 35, p. 231.

[8] Brown: Tr. Chicago Path. Soc., 1917, x, p. 184.

To test further the part of the spleen in the toxic effect, 5 rabbits were splenectomized, and 10 days later rather large doses of anilin were given subcutaneously, 0.4 c.c. for 4 days, and then 0.8 c.c. for 3 or 4 days, till the animals died. These animals stood, on the average, about twice as much anilin, and the red count was reduced about half as rapidly as in the nonsplenectomized ones. The resistance of the erythrocytes of these animals to hypnotic salt solutions did not appear to be increased. At necropsy 4 of these 5 splenectomized rabbits showed degeneration of the protoplasm of the liver cells about the central vein, and the 5th, a focal necrosis of the liver with collections of round cells and giant cell-formation.

Many of the rabbits injected with anilin were injected also with sheep blood, and the course of the resulting antisheep lysin and precipitin determined. In all cases, 25 c.c. of sheep blood were injected intraperitoneally at one time. The results indicate the when anilin is injected subcutaneously in the quantities indicated and sheep blood intraperitoneally, at about the same time, there is no restraint of antibody-formation. In 1 series in which the anilin was injected intraperitoneally and the blood also intraperitoneally, but several days later, there was marked restraint of antibody-production. In this case, questions arise as to the possible effect conditions produced by anilin in the peritoneal cavity may have had on the blood and its absorption.

CONCLUSIONS

In rabbits, anilin has a specific affinity for erythocytes, greatly diminishing the number in large doses, and in small doses causing signs of degeneration in stippling, metachromasia, and anisocytosis.

It has no effect on the leukocytes of the blood or the myelocytes of the marrow. It may cause areas of focal necrosis in the liver, and there is evidence of blood destruction in the spleen. The spleen appears to play some rôle in the toxic effect, splenectomized rabbits being more resistant and their red cells reduced less quickly than non-splenectomized rabbits. It brings about a transitory hyperplasia of the bone marrow due to an increase in the normoblasts and nongranular cells.

In rabbits, anilin does not appear to affect the production of antibodies, except possibly when both the anilin and the antigen are injected intraperitoneally.

In guinea-pigs, anilin causes a local induration and ulceration of the skin at the site of inoculation, but does not reduce the red count, although there is some evidence of blood destruction in increased pigmentation of the spleen; in this animal, also, anilin may cause focal necrosis of the liver.

As the effects of anilin in guinea-pigs is not quite the same as in rabbits, no direct conclusion may be drawn from these experiments as to its effects in man.